(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/015691 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/21323

(22) International Filing Date: 14 August 2002 (14.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/313,222 17 August 2001 (17.08.2001) US
60/383,846 28 May 2002 (28.05.2002) US

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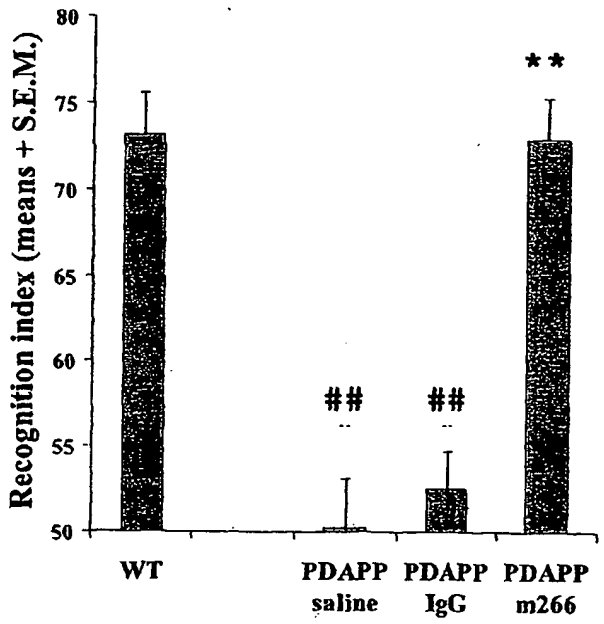
(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: RAPID IMPROVEMENT OF COGNITION IN CONDITIONS RELATED TO Aβ



(57) Abstract: The present invention is a method for effecting rapid improvement in cognition in subjects suffering from conditions or diseases related to the Aβ peptide, including Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, mild cognitive impairment, and the like. The method comprises administering anti-Aβ antibodies to the subject, especially antibodies having a high affinity for soluble forms of Aβ. X-15240

WO 03/015691 A2

European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

RAPID IMPROVEMENT OF COGNITION IN CONDITIONS RELATED TO Aβ

This application claims the priority of U.S. Provisional Application 60/313,222, filed August 17, 2001, and U.S. Provisional Application 60/383,846 filed May 28, 2002, both of which are expressly incorporated by reference.

This invention is in the field of medicine. More particularly, this invention is in the field of treatment of cognitive impairments associated with the Aβ peptide, including those involved in Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, certain vascular dementias, and certain forms of mild cognitive impairment.

The number of individuals exhibiting cognitive impairments or dementia is rising rapidly, and the rate of rise is expected to increase. Dementia afflicts an estimated 19 million people around the world. The anticipated longer life expectancy and the changing demographic distribution of age groups in the developed as well as the developing world will lead to a significant increase in the prevalence of dementia. Without significant advances in treatment, the number of people with dementia in the world will have doubled by the year 2050.

There is a strong relationship between health care costs and declines in cognitive functioning, activities of daily living (ADLs), and worsening behavioral symptoms. Early in Aβ related diseases, care is often provided at home, and costs are relatively low. But, behavioral symptoms such as aggressiveness, agitation, and incontinence often lead to a breaking point when family members are no longer able to continue to provide care. Costs thereafter escalate dramatically because the demented patient is no longer manageable at home and must be institutionalized. Medical interventions that delay institutionalization would therefore help reduce the costs of these diseases, and help to alleviate the tremendous burdens that cognitive impairment imposes on caregivers and on the subject suffering such decline.

Alzheimer's disease is by far the most common dementing disorder, accounting for roughly 60% to 80% of all dementia patients. It is a neurodegenerative disorder characterized by progressive loss of cognitive abilities and neuropathological features. The pathological aspects include neuropil and cellular disruptions in the form of amyloid deposits, neurofibrillary tangles (NFTs), oxidative stress, synapse loss, and neuritic plaques, and neuronal loss in selective brain regions (especially, the large cortical

neurons, amygdala, hippocampus, entorhinal cortex, nucleus basalis of Meynert, and locus ceruleus).

Amyloid deposits are extracellular proteinaceous deposits seen in the associative cortices and limbic system, their principal constituent being 39-43 amino acid peptide(s), the β-amyloid peptides (Aβ). Aβ derives from the processing of a larger transmembrane protein: the β-amyloid precursor protein (APP).

Over the past few years, attempts to prevent or treat AD-like neuropathology have focused on the "amyloid cascade hypothesis" of AD pathogenesis. Some therapeutic approaches have targeted enzymes that cleave APP into Aβ peptides with the aim to reduce production of the Aβ peptides. Other approaches have aimed at increasing clearance of the Aβ peptides from plaques. Among the latter approaches, immunization against Aβ has brought interesting results with regards to the prevention of amyloid deposition in mouse models of AD.

Indeed, prolonged and repeated administration of certain Aβ preparations has been shown to reduce or prevent amyloid deposition as well as the occurrence of memory impairments in mouse models of AD [Schenk, *et al., Nature.* 400:173-177 (1999); Weiner, *et al., Ann. Neurol.* 48:567-579 (2000); Janus, *et al., Nature* 408:979-982. (2000); Morgan, *et al., Nature* 408:982-985 (2000); Schenk, *et al.,* WO00/72880]. Treatments consisted of multiple, peripheral or nasal administrations of Aβ peptide in various forms.

In their study, Janus, *et al.* reported that prolonged administration of an aggregated Aβ preparation to double mutant APP TgCRND8 mice (K670N/M671L and V717F) partially prevented the development of reference memory deficits in a water maze as compared with non-immunized transgenic mice. Only a 50% reduction in the size and number of dense core amyloid deposits was observed, and this treatment had no effect on the total insoluble pool of Aβ in brain. These authors however speculated that prevention of memory deficits resulted from the reduced amyloid pathology observed in immunized mice.

Morgan, *et al.* reported similar effects of chronic administration of an Aβ preparation on memory impairment in two different strains of transgenic mice using a radial-arm water maze. The authors speculated that immunization could prevent memory

deficits by altering either the amyloid pathology or an unknown pool of non-deposited Aβ. However, this is mere speculation unsupported by the work. It cannot be concluded from those studies that the treatment altered a soluble pool of Aβ, and thereby reduced memory impairments and the amyloid pathology in parallel.

Passive immunization, consisting of prolonged peripheral administration over several weeks or months with certain anti-Aβ antibodies, also prevented the development of amyloid deposits [Bard, *et al., Nat. Med.* 6:916-919 (2000); DeMattos, *et al., Proc. Natl. Acad. Sci. USA.* 98:8850-8855 (2001); Schenk, *et al.,* WO00/72880; DeMattos, *et al.,* PCT/US/01/06191, filed February 26, 2001]. APPV717F transgenic mice (PDAPP mice) develop age-dependent object recognition memory impairments [Dodart, *et al., Behav. Neurosci.* 113:982-990 (1999)]. Treatment of PDAPP mice with weekly administration of mouse antibody 266 for seven weeks caused a significant increase in plasma Aβ and reversed these memory impairments in very old APPV717F transgenic mice, without necessarily altering the brain amyloid burden [DeMattos, *et al.,* PCT/US/01/06191, filed February 26, 2001].

The few instances in which treatments with either Aβ preparations or with anti-Aβ antibodies have caused improvement in cognition in experimental models of Alzheimer's disease all involved repeated administration over a period of many weeks to many months. Further, the use of Aβ preparations carry risk of toxicity and the development of relatively long-term and possibly adverse immunological responses. Treatments aimed at the pathology of Aβ-related conditions and diseases as described above, i.e., slowing, stopping, or reversing disease progression especially as measured by plaque, are certainly needed and are very important. However, these treatments require lengthy interventions. They are not likely to provide rapid improvement in symptoms.

Certain soluble forms of Aβ are believed to have toxic effects on neurons, including increased oxidative stress, precipitating programmed cell death, and lowering cell injury thresholds. These forms of soluble Aβ may determine the severity of neurodegeneration and/or cognitive decline [McLean, *et al., Ann. Neurol.* 46:860-866 (1999); Lambert, *et al. Proc. Nat'l Acad. Sci. (USA)* 95:6448-6453 (1998); Naslund, *J. Am. Med. Assoc.* 283:1571 (2000)]. Furthermore, evidence suggests that Aβ can be transported back and forth between brain and the blood [Ghersi-Egea, *et al., J.*

Neurochem. 67:880-883 (1996); Zlokovic, *et al. Biochem. Biophys. Res. Comm.* 67:1034-1040 (1993); Shibata, *et al., J. Clin. Invest.* 106:1489-1499 (2000)] and that Aβ in plaque may exchange with soluble Aβ in the brain and blood [Kawarabayashi, *et al., J. Neurosci.* 21:372-381 (2001)]. However, the acute effects of the various soluble forms of Aβ on cognition has not been established, nor is there any suggestion that acutely altering Aβ exchange between plaque, CSF, and blood could affect cognition in any meaningful way.

For a subject suffering from one of these conditions or diseases and for those providing care to such subject, rapid relief from the associated cognitive impairment symptoms would be of great significance. The art however does not teach or recognize any methods for obtaining rapid improvement in cognitive functioning in Aβ-related conditions and diseases.

BRIEF SUMMARY OF THE INVENTION

We remedy this deficiency by providing herein a method for effecting rapid improvement of cognition in a subject having a condition or disease related to Aβ, comprising administering to the subject an effective amount of an anti-Aβ antibody. The invention also includes the use of an anti-Aβ antibody to prepare a medicament for effecting rapid improvement of cognition in a subject having a condition or disease related to Aβ

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Object recognition memory performance 24 hours after administration of m266 anti-Aβ antibody. The recognition index is the percentage of time spent exploring a novel object during trial 2 (test trial). Both saline- and control IgG-treated tg mice performed at chance levels (recognition index = 50%), whereas m266-treated tg mice and WT mice significantly performed above chance (t-test analysis). Values are means ± SEM; ** means $p < 0.0001$ vs. saline- and IgG-treated tg groups; ## means $p < 0.0001$ vs. wild type (WT) mice.

Figure 2. Plasma Aβ40 and Aβ42 levels 24 hours after administration of m266. Plasma levels correlated with object recognition memory performance. (A) Plasma levels of both peptides are markedly increased in APP^{V717F} tg mice acutely administered m266,

compared to saline or control IgG-treated tg mice. Values are means ± SEM; (B) Bivariate scattergrams showing highly significant correlation between plasma levels of Aβ and the object recognition memory performance.

Figure 3. Apparatus used for holeboard spatial learning assay.

Figure 4. Acute Aβ antibody treatment improved reference memory in APPV717F mice.

Figure 5. Acute Aβ antibody treatment decreased total errors in APPV717F mice.

Figure 6. Correlation between Log (Aβ flux) and Log (affinity of various anti-Aβ antibodies for soluble Aβ).

Figure 7. Lack of correlation between Log (Aβ flux) and Log (affinity of various anti-Aβ antibodies for insoluble Aβ).

Figure 8. Object recognition memory performance 24 hours after administration of 266 or 3D6 anti-Aβ antibody. (* means $p<0.05$ vs. saline or IgG, *** means $p<0.001$ vs. saline or IgG).

Figure 9. Correlation between Log (Aβ flux) and Log (affinity of various anti- Aβ antibodies for soluble Aβ using altered BIAcore method).

DETAILED DESCRIPTION OF THE INVENTION

By "rapid" is meant within a very short time period, in terms of clinical relevance. Although significant responses in cognition occur within twenty-four hours of administering anti-Aβ antibodies to transgenic mice engineered to express human Aβ at high concentrations in their brains, it is not expected that cognitive responses would necessarily occur as rapidly in all other subjects. Rapid includes a period of time no more than one month. In particular subjects or with particular antibodies, rapid may mean within one day, or two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, or thirty-one days.

It is preferred that the time period within which rapid symptomatic improvement of cognition occurs is twenty-four hours. Other preferred periods for rapid symptomatic improvement of cognition are one day, two days, three days, four days, five days, six days,

seven days, one week, two weeks, three weeks, four weeks, and one month. One day and one week are more preferred periods for rapid symptomatic improvement of cognition.

By "improvement" is meant a process of making cognition more normal, nearer to a standard or to an expected level, or simply making it better in some aspect.

By "cognition" is meant short-term memory, long-term memory, abstraction, judgment, language, praxis, visuospatial skills, behavior or personality. Cognition may be assessed non-human subjects using any of a wide array of tests [Weiner, *et al., Ann. Neurol.* 48:567-579 (2000); Janus, *et al., Nature* 408:979-982. (2000); Morgan, *et al., Nature* 408:982-985 (2000); Dodart, *et al., Neuroreport.* 8:1173-1178 (1997)]. Cognition in humans may be assessed using any of the tests mentioned below, plus tests such as the Alzheimer's Disease Assessment Scale – Cognitive subscale (ADAS-Cog) component [Rosen, *et al., Am J Psychiatry* 141: 1356–1364 (1984)]. The ADAS-Cog is a 70-point test that briefly assesses memory, language capabilities, and other cognitive functions in patients with dementia.

By "subject" is meant a mammal, preferably a human. A subject will benefit from the present invention if the subject has a cognitive deficiency or aberration caused by or related to the presence of toxic forms and/or concentrations of soluble Aβ in the subject's brain. Even though the nature or concentration of Aβ in a subject's brain may not be known with certainty, the administration of well-known tests of cognition in subjects who are suspected or known to suffer from an Aβ-related disease will suffice to identify many subjects who will benefit from the present methods. For other subjects, a combination of clinical assessment, subject history, and perhaps laboratory or other diagnostic assessments may be needed to identify subjects likely to benefit from the present invention.

A mental status examination of cognitive domains such as language, memory, visuospatial function, executive function (ability to manipulate previously acquired information, multitasking, abstraction, judgment, calculation, etc.), personality, and mood will aid in identifying subjects most likely to benefit from the present invention. Subjects meeting the criteria for a diagnosis of probable AD (i.e., dementia, 40-90 years old, cognitive deficits in two or more cognitive domains, progression of deficits for more than six months, consciousness undisturbed, and absence of other reasonable diagnoses) will

benefit from the present invention. Likewise, subjects with Down's syndrome will benefit from the present invention.

The Mini-Mental State Examination (MMSE) is widely used, with norms adjusted for age and education [Folstein *et al, J. Psych. Res.* 12:196-198 (1975); Anthony, *et al., Psychological Med.* 12: 397-408 (1982); Cockrell, *et al., Psychopharmacology* 24: 689-692 (1988); Crum, *et al., J. Am. Med. Assoc'n* 18:2386-2391 (1993)]. The MMSE is a brief, quantitative measure of cognitive status in adults. It can be used to screen for cognitive impairment, to estimate the severity of cognitive impairment at a given point in time, to follow the course of cognitive changes in an individual over time, and to document an individual's response to treatment. Cognitive assessment of subjects may require formal neuropsychologic testing, with follow-up testing separated by nine months or more (in humans). The "cognitive symptoms" treated by the present invention are cognitive deficits known to be associated with conditions and diseases related to Aβ as discussed herein.

Laboratory assessment or structural imaging studies may identify reversible causes of cognitive impairment, which are not likely to respond to the present invention, and to identify focal lesions, significant white matter disease implicating vascular dementia, significant temporal atrophy. In AD, functional studies such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) typically show abnormalities most marked in the parietal and temporal lobes bilaterally. These studies are particularly useful in differentiating early AD from normal aging or frontotemporal degeneration. They are not required to identify subjects who are likely to benefit from the present invention. The combination of medial temporal atrophy determined by structural imaging and parietal impairment determined with functional imaging is, however, a useful biomarker of AD. MRI can be used to exclude subjects with significant cerebrovascular disease.

ApoE genotyping is not useful in isolation, but may increase the specificity of the diagnosis when patients do not have the E4 allele if the diagnosis is in question. Another potential biomarker is the combined assessment of cerebral spinal fluid (CSF) Aβ42 and tau concentrations. A low Aβ42 and high tau concentration have a high predictive value (90%) and negative predictive value (95%) based on a clinical diagnosis of probable AD.

By "condition or disease related to Aβ" is meant conditions and diseases that are associated with: 1) the development of β-amyloid plaques in the brain, 2) the synthesis of abnormal forms of Aβ, 3) the formation of particularly toxic forms of Aβ, or 4) abnormal rates of synthesis, degradation, or clearance of Aβ. Conditions and diseases such as Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, certain vascular dementias, and mild cognitive impairment are known or suspected of having relationship to Aβ.

Alzheimer's disease, discussed above, is the most prevalent disease related to Aβ (60-80% of dementias). Definite diagnosis of AD is only possible presently with a post-mortem examination. But, a diagnosis of probable AD correlates highly with AD pathology. Vascular dementia (VaD), dementia with Lewy bodies (DLB), and frontotemporal dementia (FTD) together probably account for 15% to 20% of dementias, with other disorders (e.g., hydrocephalus; vitamin B12 deficiency) accounting for about 5%. Of these, only certain vascular dementias are suspected of having a significant Aβ component.

A state of increased risk or early manifestation of cognitive problems that often progresses to AD is termed mild cognitive impairment (MCI). MCI is a clinical entity characterized by memory loss, without significant dysfunction in other cognitive domains and without impairment in activities of daily living (ADL) function. Early diagnosis and treatment of MCI, including with the use of the present invention, is important. Currently the best predictor of preclinical AD is a diagnosis of MCI, because 30-50% of subjects with MCI develop AD within 3-5 years. One structural correlate of MCI that may be predictive for which subjects will develop AD is the volume of the hippocampus. Subjects with MCI have smaller hippocampi than age-equivalent controls and appear to experience atrophy of the structure at a more rapid pace.

By "administering" is meant the act of introducing a pharmaceutical agent into the subject's body. The parenteral route is the preferred route of administering the antibodies in the methods of the present invention. Preferred parenteral routes include subcutaneous, intravenous, and intraperitoneal.

By "effective dose" is meant an amount of antibody, which when administered to the subject, will cause rapid improvement in cognition. The amount of antibody in an

effective dose can be readily determined by a skilled physician or clinical pharmacologist, taking into account the subject's body mass, age, gender, severity of the Aβ-related condition or disease, affinity of the antibody for soluble Aβ, route of administration, and similar factors well known to physicians and pharmacologists. Effective doses may be expressed, for example, as the total mass of antibody (e.g., in grams, milligrams or micrograms) or as a ratio of mass of antibody to body mass (e.g., as grams per kilogram (g/kg), milligrams per kilogram (mg/kg), or micrograms per kilogram (mg/kg). An effective dose of antibody in the present methods will range between 1 μg/kg and 100 mg/kg. A more preferred range for effective dose in the present invention is between 1 mg/kg and 30 mg/kg. Yet more preferred ranges are between 1 μg/kg and 10 mg/kg, 1 μg/kg and 10 mg/kg, between 1 μg/kg and 1 mg/kg, between 1 μg/kg and 0.3 mg/kg, and between 1 μg/kg and 0.1 mg/kg.

"Aβ peptide" and "Aβ" refer to a peptide that is derived from amyloid precursor protein ("APP" – Alzheimer's disease amyloid A4 protein [Precursor]) by proteolytic cleavage. Full-length Aβ peptides are from 39 to 43 amino acids long in humans, for example. Full length Aβ peptide may undergo further cleavage *in vivo* to produce Aβ fragments that are shorter at the N-terminus, at the C-terminus, or both, by one to several amino acids. Soluble full-length Aβ peptide or fragments thereof may be used as antigens to raise antibodies that bind soluble Aβ peptide with high specificity and affinity. For example, among the many Aβ peptide fragments used for this purpose, the Aβ 13 – 28 fragment (conjugated via m-maleimidobenzoyl-N-hydroxysuccinimide ester to an anti-CD3 antibody) was used to raise antibody 266 [Seubert, *et al., Nature* 359:325 – 327 (1992)]. The use of antibody 266 for selective measurement of soluble Aβ is well known [Schenk, *et al.,* U.S. Patents 5,593,846, 5,766,846, 5,872,672, and 6,284,221 B1]. Assessment of binding to "soluble Aβ" is carried out with Aβ in an unaggregated form, predominantly monomeric form, as described hereinbelow.

The expression "anti-Aβ antibody" means an antibody that binds to soluble Aβ. The antibody preferably binds with high affinity to soluble Aβ. Affinity higher than that of antibody 266 is preferred. Affinity higher than 10^{-9} M is preferred. Affinity higher than 10^{-10} M is more preferred. Affinity higher than 10^{-11} M is yet more preferred. Affinity higher than 10^{-12} M is highly preferred. The term "Aβ" in this context includes the 39, 40, 41, 42, and 43 amino acid peptides derived from the APP protein in vivo by

proteolysis, and any fragments of those peptides, such as N-terminally shortened peptides derived from those peptides (e.g., denoted by, for example, x-42, where x = 1, 2, 3, etc.), C-terminally shortened peptides derived from 1-39, 40, 41, and 42 peptides, and peptides shortened at both termini. The expression "Aβ40" is used to denote peptides that bind to antibodies that bind only at an Aβ C-terminus that ends at position 40. The expression "Aβ42" denotes peptides that bind to antibodies that bind only at an Aβ C-terminus that ends at position 42.

By "affinity" is meant the strength of the binding of a single antigen-combining site with an antigenic determinant. It is a measure of the binding strength between antibody and a simple hapten or antigen determinant. It depends on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and on the distribution of charged and hydrophobic groups. It includes the concept of "avidity," which refers to the strength of the antigen-antibody bond after formation of reversible complexes. The most direct way of measuring antibody affinity is by the well known method of equilibrium dialysis. Methods requiring less time or materials than equilibrium dialysis are known, for example, the method of Griswold, *et al. Immunology Letters* 9:15-18 (1985) and the kinetic BIAcore method described herein. The BIAcore method relies on the phenomenon of surface plasmon resonance (SPR), which occurs when surface plasmon waves are excited at a metal/liquid interface. Light is directed at, and reflected from, the side of the surface not in contact with sample, and SPR causes a reduction in the reflected light intensity at a specific combination of angle and wavelength. Bimolecular binding events cause changes in the refractive index at the surface layer, which are detected as changes in the SPR signal.

The dissociation constant, KD, and the association constant, KA, are quantitative measures of affinity. At equilibrium, free antigen (Ag) and free antibody (Ab) are in equilibrium with antigen-antibody complex (Ag-Ab), and the rate constants, ka and kd, quantitate the rates of the individual reactions:

$$Ag + Ab \underset{kd}{\overset{ka}{\rightleftharpoons}} Ag\text{-}Ab$$

At equilibrium, ka [Ab][Ag] = kd [Ag-Ab]. The dissociation constant, KD, is given by: KD = kd/ka = [Ag][Ab]/ [Ag-Ab]. KD has units of concentration, most typically M, mM, mM, nM, pM, etc. When comparing antibody affinities expressed as KD, having greater affinity for Aβ is indicated by a lower value. The association constant, KA, is given by: KA = ka/kd = [Ag-Ab]/ [Ag][Ab]. KA has units of inverse concentration, most typically M^{-1}, mM^{-1}, μM^{-1}, nM^{-1}, pM^{-1}, or the like. When comparing antibody affinities expressed as KA, having greater affinity for Aβ is indicated by a higher value. "Affinity for soluble" Aβ is measured as described herein using samples of Aβ, typically Aβ1-40 or Aβ1-42, that are reasonably free of aggregated forms of Aβ. For antibodies having high affinity for soluble Aβ, particular care must be taken when using the BIAcore technology, as described hereinbelow.

By "antibody" is meant a whole antibody, including without limitation an animal-derived antibody (e.g., murine), chimeric, humanized, human sequence, recombinant, transgenic, grafted and single chain antibody, and the like, and any fusion proteins, conjugates, fragments, or derivatives thereof. An antibody comprises protein resembling an antibody in the broadest sense in that the protein comprises a binding site for an antigen, which binding site is comprised of three pairs of complementarity determining regions. Antibody includes a whole immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, or an immunologically effective fragment of any of these. An antibody fragment, or simply fragment, means an Fv, a disulfide linked Fv, scFv, Fab, Fab', or $F(ab')_2$ fragment, which terms are well known in the art. In some contexts herein, fragments will be mentioned specifically for emphasis. Nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as a protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred under some circumstances, such as when prolonged residence in the body is desirable, or when minimum risk of developing neutralizing antibodies. Antibodies, except perhaps for certain types in which cross-linking between

chains is accomplished by peptide or other chemical chains, are properly cross-linked via disulfide bonds.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. IgG isotypes are preferred. Of the IgG subclasses, IgG1 and IgG4 are preferred.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact non-fragment antibody and certain fragments (e.g., an $F(ab')_2$ fragment) has two binding sites, whereas, most fragments have only one binding site per molecule. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The framework regions align the CDRs from the two chains of each pair, enabling binding to a specific epitope. From N- terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat, *et al.*, "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, *et al., J. Mol. Biol.* 196:901-917 (1987); Chothia, *et al., Nature* 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). Humanized antibodies are also referred to as CDR-grafted or reshaped antibodies. A humanized immunoglobulin does not encompass a chimeric antibody having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the

corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Although the mechanics of producing an engineered, humanized mAb using the techniques of molecular biology are relatively straightforward, simple grafting of xenogenic (usually rodent) complementarity-determining regions (CDRs) into human frameworks does not always reconstitute the binding affinity and specificity of the original mAb. The design of the engineered mAb is the critical step in reproducing the function of the original molecule. This design includes various choices: the extents of the CDRs, the human frameworks to use and the substitution of residues from the rodent mAb into the human framework regions (backmutations). The positions of these backmutations have been identified principally by sequence/structural analysis or by analysis of homology models of the variable regions' 3D structure. Recently, phage libraries have been used to vary the amino acids at chosen positions. Similarly, many approaches have been used to choose the most appropriate human frameworks in which to graft the rodent CDRs. Variable regions with high amino acid sequence identity to the rodent variable regions (homology matching or best-fit), consensus or germline sequences, or fragments of the framework sequences within each light or heavy chain variable region from several different human mAbs may be used. Alternatively the surface rodent residues may be replaced with the most common residues found in human mAbs ("resurfacing" or "veneering").

The design of humanized immunoglobulins starting from a non-human antibody that has properties found to be critical in the present invention may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used (acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor immunoglobulin):

(a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, *et al., Proc. Nat'l. Acad. Sci. (USA)* 86:10029-10033 (1989); Co, *et al., Proc. Nat'l. Acad. Sci. (USA)* 88:2869 (1991)]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

Human antibodies may be readily obtained using known methods, such as, from human immune donors, from phage libraries, and from transgenic animals such as mice. Antibodies may be rescued from immune human donors using either EBV transformation of B-cells or by PCR cloning and phage display. Synthetic phage libraries may be created that use randomized combinations of synthetic human antibody V-regions. By selection on antigen, so called 'fully human antibodies' can be made, in which it is assumed that the V-regions are very human-like in nature. Transgenic mice can be created that have a repertoire of human immunoglobulin germline gene segments. These mice, when immunized with soluble Aβ, make human antibodies directed against soluble Aβ.

Preparation of high affinity humanized or human antibodies for use in the present invention may be carried out by methods well known in the art, including preparing monoclonal antibodies using well known techniques and screening for high affinity antibodies, or by first identifying a monoclonal antibody having reasonably high affinity and then improving the affinity using well known methods such as those described, for example, in: US Patent Nos. 5,976,562, 5,824514, 5,817,483, 5,814,476, 5,763,192, 5,723,323; WO97/29131; Thomas, *et al., J. Biol. Chem.* 277:2059-2064 (2002); Shreder, *Methods* 20:372-379 (2000); Boder, *et al., Proc. Nat'l Acad. Sci. (USA)* 97:10701-10705 (2000); Chen, *et al., J. Mol. Biol.* 293:865-881 (1999); Wu, *et al., Proc. Nat'l. Acad. Sci. (USA)* 95:6037-6042 (1998); Hoogenbloom, *Trends Biotechnol.* 15:62-70 (1997); Rader,

et al., Curr. Opin. Biotechnol., 8:503-508 (1997); Crameri, *et al., Nature Medicine* 2:100-102 (1996); Crameri, *et al., Nature Medicine* 2:100-103 (1996); Schier, *et al., J. Mol. Biol.* 255:28-43 (1996); Yang, *et al., J. Mol. Biol.* 254:392-403 (1995); Yang, *et al., J. Mol. Biol.* 254:392-403 (1995); Yelton, *et al., J. Immunol.* 155:1994-2004 (1995); Stemmer, *Proc. Nat'l. Acad. Sci. (USA)* 91:10747-10751 (1994); Stemmer, *Nature* 370:389-391 (1994); Huse, *et al., Internat'l Rev. Immunol.* 10:129-137 (1993); the portion of each of which having to do with preparation of high affinity antibodies is incorporated herein by reference.

The antibodies used in the present invention will most advantageously be expressed in recombinant hosts and purified using well known techniques [Page, *et al., Bio/Technol.* 9, 64-68 (1991); Carroll, *et al., Mol. Immunol.* 29, 821-827 (1992); Coloma, *et al., J. Immunol. Meth.* 152, 89-104 (1992); Bebbington, *et al., Bio/Technol.* 10, 169-175 (1992); Deyev, *et al., FEBS Lett.* 330, 111-113 (1993); Bender, *et al., Hum. Antibodies Hybridomas* 4, 74-79 (1993); Norderhaug, *et al., J. Immunol. Meth.* 204, 77-87 (1997); Poul, *et al., Eur. J. Immunol.* 25, 2005-2009 (1995), each of which is incorporated herein by reference].

A preferred antibody for use in the present invention is an antibody that binds to the same epitope on Aβ that 266 binds or any antibody that competitively inhibits the binding of 266 and human or mouse Aβ. The skilled reader will know how to determine, using well known methods, whether any particular antibody competitively inhibits the binding of 266 and human Aβ. For example, a competitive ELISA method could be used. Wells of a 96-well ELISA plate (e.g., Nunc-Immuno plate, Cat # 439454, NalgeNunc) are coated with Aβ peptide (1-40 is particularly convenient, but other lengths could be used also), optionally conjugated to a larger protein such as albumin. After washing the wells, they are blocked as appropriate, and then rinsed and dried appropriately. A mixture of biotinylated 266 antibody (e.g., biotinylated humanized 266, having as light chain the amino acid sequence of SEQ ID NO:11 and as heavy chain the amino acid sequence of SEQ ID NO:12) at 0.3 μg/ml final concentration, for example, and a competitor antibody (starting at 750 μg/ml final concentration and serial 3-fold dilutions) are added in a final volume of 100 μl per well. No-competitor and background controls are run. The ELISA plate is incubated at an appropriate temperature for an appropriate length of time, and then the wells are washed. After washing the wells, HRP-conjugated streptavidin (Cat #

21124, Pierce), or equivalent, is added to each well (e.g., 100 µl of 1 µg/ml). The plate is incubated at room temperature for an appropriate length of time, e.g., 30 min, and then is thoroughly washed. For color development, 100 µl/well of ABTS Peroxidase Substrate (Kirkegaard & Perry Laboratories), or equivalent, is added. Color development is stopped and absorbance is read (e.g., at 415 nm). The absorbances are plotted against the log of the competitor concentration, curves are fitted to the data points (e.g., using Prism or equivalent) and the IC50 determined using methods well known in the art. An antibody having an IC50 greater than 100-fold less than of that of 266 is considered to competitively inhibit the binding of 266 to Aβ. The affinity of an antibody for soluble Aβ can be determined using methods well known in the art or described herein.

Antibody 266 has the following amino acid sequences as CDRs:

light chain CDR1:

```
 1               5                   10                  15
Arg Ser Ser Gln Ser Leu Ile Tyr Ser Asp Gly Asn Ala Tyr Leu His
(SEQ ID NO:1)
```

light chain CDR2:

```
 1               5
Lys Val Ser Asn Arg Phe Ser  (SEQ ID NO:2)
```

light chain CDR3:

```
1               5
Ser Gln Ser Thr His Val Pro Trp Thr  (SEQ ID NO:3)
```

heavy chain CDR1:

```
 1               5
Arg Tyr Ser Met Ser (SEQ ID NO:4)
```

heavy chain CDR2:

```
 1               5                   10                  15
Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val Lys Gly
(SEQ ID NO:5)
```

and, heavy chain CDR3:

```
 1
Gly Asp Tyr  (SEQ ID NO:6).
```

In humanized versions of 266, human framework regions may optionally have substitutions of one to several residues from mouse 266 for the purpose of maintaining the strength or specificity of the binding of humanized antibody 266 [WO01/62801]. A preferred light chain variable region of a humanized 266 antibody has the following amino acid sequence:

```
1               5                  10                  15
Asp Xaa Val Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Xaa Gly

            20                  25                  30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Xaa Tyr Ser

        35                  40                  45
Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser

    50                  55                  60
Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

65                  70                  75                  80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

                85                  90                  95
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Ser Gln Ser

            100                 105                 110
Thr His Val Pro Trp Thr Phe Gly Xaa Gly Thr Xaa Xaa Glu Ile Lys

Arg                 (SEQ ID NO:7)
```

wherein:

Xaa at position 2 is Val or Ile;

Xaa at position 7 is Ser or Thr;

Xaa at position 14 is Thr or Ser;

Xaa at position 15 is Leu or Pro;

Xaa at position 30 is Ile or Val;

Xaa at position 50 is Arg, Gln, or Lys;

Xaa at position 88 is Val or Leu;

Xaa at position 105 is Gln or Gly;

Xaa at position 108 is Lys or Arg; and

Xaa at position 109 is Val or Leu.

A preferred heavy chain variable region of a humanized 266 antibody has the following amino acid sequence:

```
 1               5                  10                  15
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly Gly

            20                  25                  30
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr

        35                  40                  45
Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val

    50                  55                  60
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
65                  70                  75                  80
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr

                85                  90                  95
Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys

            100                 105                 110
Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser

                        (SEQ ID NO:8)
```

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

A particularly preferred light chain variable region of a humanized 266 antibody has the following amino acid sequence:

```
1               5                  10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

            20                  25                  30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile Tyr Ser

        35                  40                  45
Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser

    50                  55                  60
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

65                  70                  75                  80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

                85                  90                  95
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser

            100                 105                 110
Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

Arg                (SEQ ID NO:9).
```

A particularly preferred heavy chain variable region of a humanized 266 antibody has the following amino acid sequence:

```
 1              5                  10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

            20                  25                  30
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr

        35                  40                  45
Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
```

```
     50                  55                  60
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val

 65                  70                  75                  80
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr

                 85                  90                  95
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

            100                 105                 110
Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
```

(SEQ ID NO:10).

A preferred light chain for a humanized 266 antibody has the amino acid sequence:

```
 1               5                  10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu

                20                  25                  30
Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile

                35                  40                  45
Tyr Ser Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro

                50                  55                  60
Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe

                65                  70                  75
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp

                80                  85                  90
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val

                95                 100                 105
Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gln
```

110 115 120
Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val

125 130 135
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala

140 145 150
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

155 160 165
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

170 175 180
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu

185 190 195
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys

200 205 210
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val

215
Thr Lys Ser Phe Asn Arg Gly Glu Cys (SEQ ID NO:11).

A preferred heavy chain for a humanized 266 antibody has the amino acid sequence:

1 5 10 15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

20 25 30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

35 40 45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

50 55 60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr

65 70 75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

80 85 90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

95 100 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

110 115 120
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

125 130 135
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala

140 145 150
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr

155 160 165
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

170 175 180
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val

185 190 195
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys

200 205 210
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val

215 220 225
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro

230 235 240
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro

245 250 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr

260 265 270
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe

275 280 285
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

290 295 300
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val

305 310 315
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

320 325 330
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

335 340 345
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr

350 355 360
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu

365 370 375
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

380 385 390
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro

395 400 405
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

410 415 420
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

425 430 435
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

440
Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:12).

A preferred antibody for use in the present invention is an analog of 266, in which an N-glycosylation site within CDR2 of the heavy chain (SEQ ID NO:5) is engineered so as not to be glycosylated. Such an analog has higher affinity for A□ than 266 does, and

comprises a light chain and a heavy chain, wherein the light chain comprises the three light chain complementarity determining regions (CDRs) from mouse monoclonal antibody 266 (SEQ ID NO:1-3), and wherein the heavy chain comprises heavy chain CDR1 and CDR3 from mouse monoclonal antibody 266 (SEQ ID NO: 4 and 6, respectively), and a heavy chain CDR2 having the sequence given by SEQ ID NO:13:

```
 1               5                  10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys Gly
```

(SEQ ID NO:13)

wherein,

Xaa at position 7 is any amino acid, provided that if Xaa at position 8 is neither Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

Xaa at position 8 is any amino acid, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro; and

Xaa at position 9 is any amino acid, provided that if Xaa at position 7 is Asn and Xaa at position 8 is neither Asp nor Pro, then Xaa at position 9 is neither Ser nor Thr.

By "any amino acid" is meant any naturally occurring amino acid. Preferred naturally-occurring amino acids are Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr.

A preferred group of antibodies are those having as light chain CDR1-CDR3 the sequences SEQ ID NO:1-3, respectively, as heavy chain CDR1 and CDR3 the sequences SEQ ID NO:4 and 6, respectively, and wherein the sequence of heavy chain CDR2 is SEQ ID NO:13, wherein:

Xaa at position 7 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 8 is neither Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

Xaa at position 8 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro; and

Xaa at position 9 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 8 is neither Asp nor Pro, then Xaa at position 9 is neither Ser nor Thr.

Another description of the preferred group is: antibodies or fragments thereof having as light chain CDR1-CDR3 the sequences SEQ ID NO:1-3, respectively, as heavy chain CDR1 and CDR3 the sequences SEQ ID NO:4 and 6, respectively, and wherein the sequence of heavy chain CDR2 is selected from the group consisting of:

1) SEQ ID NO:14

```
 1               5                   10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

Gly  (SEQ ID NO:14)
```

wherein:

Xaa at position 7 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

Xaa at position 8 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Xaa at position 9 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

2) SEQ ID NO:15

```
 1               5                   10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

Gly  (SEQ ID NO:15)
```

wherein:

Xaa at position 7 of SEQ ID NO:15 is Asn;

Xaa at position 8 of SEQ ID NO:15 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Xaa at position 9 of SEQ ID NO:15 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp, and Tyr; and

3) SEQ ID NO:16

```
 1               5                  10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

Gly  (SEQ ID NO:16)
```

wherein:

Xaa at position 7 of SEQ ID NO:16 is Asn;

Xaa at position 8 of SEQ ID NO:16 is selected from the group consisting of Asp and Pro; and

Xaa at position 9 of SEQ ID NO:16 is selected from the group consisting of Ser and Thr.

Preferred sequences for CDR2 of the heavy chain include those in which only a single amino acid is changed, those in which only two amino acids are changed, or all three are changed. It is preferred to replace Asn at position 7, or to replace Thr at position 9, or to replace both. Conservative substitutions at one, two, or all three positions are preferred. The most preferred species are those in which Asn at position 7 is replaced with Ser or Thr.

Preferred deglycosylated 266 antibodies for use in the present invention are those in which in CDR2 of the heavy chain (i.e., within SEQ ID NO:13, as described above):

Xaa at position 7 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

Xaa at position 8 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 7 is Asn, then Xaa at position 9 is neither Ser nor Thr.

An alternate description of preferred declycogsylated 266 antibodies is: antibodies or fragments thereof having as light chain CDR1-CDR3 the sequences SEQ ID NO:1-3, respectively, as heavy chain CDR1 and CDR3 the sequences SEQ ID NO:4 and 6, respectively, and wherein the sequence of heavy chain CDR2 is selected from the group consisting of:

1) SEQ ID NO:17

```
 1               5                  10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

Gly  (SEQ ID NO:17)
```

wherein:

Xaa at position 7 of SEQ ID NO:17 is selected from the group consisting of Ala, Gly, His, Gln, Ser, and Thr;

Xaa at position 8 of SEQ ID NO:17 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 of SEQ ID NO:17 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

2) SEQ ID NO:18

```
 1               5                  10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

Gly  (SEQ ID NO:18)
```

wherein:

Xaa at position 7 of SEQ ID NO:18 is Asn;

Xaa at position 8 of SEQ ID NO:18 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 of SEQ ID NO:18 is selected from the group consisting of Ala, Gly, His, Asn, and Gln.

A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:7 and a heavy chain variable region of SEQ ID NO:19:

```
 1               5                  10                  15
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Xaa Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

                65                  70                  75
Pro Asp Xaa Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa

                80                  85                  90
Xaa Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp

                95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

               110
Thr Xaa Val Thr Val Ser Ser                    (SEQ ID NO:19)
```

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:9 and a heavy chain variable region of SEQ ID NO:20:

```
1               5                   10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

                65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

                80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

                95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

                110
```

Thr Leu Val Thr Val Ser Ser (SEQ ID NO:20).

wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:11 and a heavy chain given by SEQ ID NO:21:

```
  1               5                  10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

            35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

                65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

                80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

                95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

            110                 115                 120
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

            125                 130                 135
```

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala

140 145 150

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr

155 160 165

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

170 175 180

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val

185 190 195

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys

200 205 210

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val

215 220 225

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro

230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro

245 250 255

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr

260 265 270

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe

275 280 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

290 295 300

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val

305 310 315

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

320 325 330

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

335 340 345
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr

350 355 360
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu

365 370 375
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

380 385 390
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro

395 400 405
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

410 415 420
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

425 430 435
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

440
Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:21)

wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

Preferred deglycosylated 266 antibodies having the heavy variable region according to SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 are those wherein:

Xaa at position 56 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 58 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 58 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 56 is Asn, then Xaa at position 58 is neither Ser nor Thr.

Preferred sequences for CDR2 (positions 56, 57, and 58) of the heavy chain SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 include those in which only a single amino acid is changed, those in which only two amino acids are changed, or all three are changed. It is preferred to replace Asn at position 56. It is preferred to replace Thr at position 58 with an amino acid other than Ser. It is preferred to not destroy the N-glycosylation site in the CDR2 of the 266 heavy chain by replacing Ser at position 57 with Pro or Asp. Conservative substitutions at one, two, or all three positions are preferred. The most preferred species are those in which Asn at position 56 is replaced with Ser or Thr. Particularly preferred antibodies are those in which Ser or Thr is at position 56, Ser is at position 57, and Thr is at position 58 of SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21.

The most preferred species are antibodies comprising a light chain of SEQ ID NO:11 and a heavy chain of SEQ ID NO:21, wherein in SEQ ID NO:21, Xaa at position 56 is Ser, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56S"), or wherein in SEQ ID NO:21, Xaa at position 56 is Thr, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56T").

The antibodies (including immunologically reactive fragments) are administered to a subject as identified above using standard parenteral, peripheral administration techniques, by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. The preferred routes of administration are intravenous, subcutaneous, and intraperitoneal.

The preparation of an acceptable pharmaceutical preparation of the antibodies used in the present invention, including its strength, excipients, pH, isotonicity, presentation, dosage form, and the like, is well known to the skilled person. Pharmaceutical compositions for use in the present invention should be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as

appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners. Pharmaceutical preparations for use in the present invention should be sterile or at least nearly so, and if necessary preserved or rendered bacteriostatic.

The following examples are intended to illustrate but not to limit the invention. The examples describe experiments conducted in murine systems, thus the use of murine monoclonal antibodies is satisfactory. However, in the treatment methods of the invention intended for human use, humanized or fully human antibodies are preferred.

EXAMPLES

Example 1

Rapid Improvement in Cognition after Administration of Anti-Aβ Antibody 266

APPV717F transgenic mice (PDAPP mice, eleven month old) were used [Games, *et al., Nature* 373:523-527 (1995)]. The mice were handled daily 5 days before the behavioral testing. All animals had free access to food and water. They were housed at a room temperature of 23 ± 1°C and with a light-dark cycle of 12:12 h with lights on at 6:00 a.m. Behavioral experiments were conducted during the light period, between 8:00 a.m. and 2 p.m.

The object recognition task is based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one [Ennaceur et Delacour, *Behavioral Brain Research.* 31:47-59 (1988); Dodart *et al., Neuroreport.* 8:1173-1178 (1997)]. This task was performed in a black PlexiglasTM open field (50 X 50 X 40 cm). The objects to be discriminated were a marble (1.5 cm diameter) and a plastic dice (1.8 cm edge). After each trial, the objects were handled with disposable gloves and immersed in alcohol to eliminate olfactory cues. Before experiments, several male mice were placed in the open field in order to condition the testing environment. On the first day of testing, mice were submitted to a familiarization session by placing them in the empty open field for 30 min and the distance traveled (cm) was recorded by at 5-minute intervals using a computer-assisted video tracking system (San Diego Instrument, CA). On the following day, mice were submitted to two 10 min trials with a 3 hour inter-trial delay. During trial 1, mice

were allowed to explore the open field in the presence of object A (marble or dice). The distance traveled (cm) and the time spent exploring the object (nose pointing toward the object at a distance ≤ 1 cm) were recorded with the video tracking system and by hand, respectively. For trial 2, mice were allowed to explore the open field in the presence of two objects: the familiar object ("object A") and a novel object ("object B") (e.g., a marble and a die).

A recognition index calculated for each animal was expressed by the ratio $(t_B \times 100)/(t_A + t_B)$ where t_A and t_B are the time spent on object A and object B respectively. An observer blind to the treatment status of the animals recorded the object exploration time.

Murine anti-Aβ antibody 266 ["m266", Seubert, *et al.*, *Nature* 359:325-327 (1992)] and a purified mouse IgG1 isotype control (Pharmingen) were diluted from stock solutions in PBS prior to administration. Animals were injected (500 µL, i.p.) with m266 (n=8, 1 mg/mL) control IgG solution (n=8, 1 mg/mL) or PBS (n=7) 3 hours before the familiarization session, which corresponds to 24 hours before trial 1. An additional control group of wild-type mice (WT) animals was tested in parallel in the object recognition task (n=6).

After completion of trial 2, blood and CSF fluids were sampled, and brains were processed using a 3-step extraction procedure. The first step consisted of homogenizing samples in cold PBS and complex of proteinase inhibitors (Complete™, Boehringer-Mannheim, IN) followed by centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was considered as the PBS "soluble" pool. The second step consisted of re-suspension of the pellet in RIPA (50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP40, 0.1% SDS and Complete™, pH 8.0) followed by centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was designated the "detergent soluble" pool. Finally, the third step consisted of re-suspension of the pellet in 5M Guanidine-HCl, rocking the tubes for 2 hours at room temperature, followed by centrifugation at 10,000 rpm for 10 min at 4°C. This step produced the "insoluble" pool. Aβ40 and Aβ42 were quantified in each pool using an ELISA [Bales, *et al.*, *Proc. Natl. Acad. Sci. USA.* 96:15233-15238 (1999)].

Briefly, for the ELISA, the monoclonal antibodies 2G3 and 21F12 were used to capture Aβ peptides terminating at residues 40 and 42 respectively [Johnson-Wood, *et al.*,

1997]. Biotinylated 3D6, which recognizes the Aβ 1-5 region, was used as the reporter antibody.

Proteins of plasma and CSF samples were separated by electrophoresis under non-denaturing conditions utilizing a 4-20% TBE gel (Criterion gel, Bio Rad, CA) and transferred in CAPS buffer (CAPS 10 mM, 0.01% SDS, 1% Methanol, pH 11) onto PVDF membrane. After a 1-hour block in SuperBlock blocking buffer (Pierce, IL), the membrane was probed with biotinylated 3D6 (0.045 mg/ml), thereafter reacted with StreptAvidin (1:200000) and visualized utilizing SuperSignal West Femto (Pierce, IL).

To compare behavioral data as well as the Aβ levels between groups, one-way or two-way analyses of variance (ANOVA) and correlation analyses were performed using the Statview5 software (SAS Institute Inc., NC).

As shown in Fig. 1, the object recognition index differed significantly between treatment groups (group effect: $F_{3,25} = 25.085$, $p < 0.0001$). Performance of m266-treated tg mice were significantly higher than performance of saline-treated tg mice and control IgG-treated tg mice ($p < 0.0001$) and comparable to WT mice. The object recognition index did not differ between saline- and control IgG-treated tg mice. Moreover, a within-group t-test analysis confirmed that m266- (t-value = 9.526, $p < 0.0001$) treated tg mice and WT mice (t-value = 9.581, $p < 0.0002$) performed above chance levels (50%) whereas saline- and control IgG-treated tg mice did not (t-values = 0.081 and 1.157 respectively, NS). A group effect analysis also confirmed that there were no differences between groups in total object exploration time during trial 1 ($F_{3,25} = 0.555$, NS) or trial 2 ($F_{3,25} = 0.679$, NS). The distance traveled also did not differ between groups during the familiarization session (group x block interaction: $F_{15,125} = 1.455$, NS; group effect: $F_{3,25} = 1.200$, NS), during trial 1 (group effect: $F_{3,25} = 1.326$, NS) or during trial 2 (group effect: $F_{3,23} = 1.334$, NS).

Following administration of m266, as detected by ELISA (Fig.2a), the levels of both Aβ40 and Aβ42 peptides were significantly increased in plasma samples when compared to saline- and IgG-treated mice ($p < 0.0001$). The increase for Aβ40, when compared to saline-treated mice was approximately 150-fold and the increase for Aβ42, was approximately 70-fold. Neither Aβ40 nor Aβ42 levels differed between saline- and control IgG-treated mice. Remarkably, the plasma levels of both Aβ peptides following

m266 treatment were highly correlated with object recognition memory performance ($r = 0.774$, $p < 0.0001$ for Aβ40; $r = 0.781$, $p < 0.0001$ for Aβ42) (Fig.2b).

In CSF samples, levels of Aβ40 significantly differed between groups ($F_{2,19} = 4.798$, $p < 0.05$), m266-treated animals showing increased levels of this peptide compared to saline- or control IgG-treated animals ($p < 0.05$, levels of Aβ40 in ng per mL of protein: saline, 7.79 ± 1.73; control IgG, 8.72 ± 2.9; m266, 16.57 ± 3.25). No difference in CSF levels of Aβ42 was observed between groups ($F_{2,19} = 3.006$, NS).

In the cerebral cortex, a significant difference between groups was observed only for Aβ40 in the PBS-soluble pool ($F_{2,20} = 7.785$, $p < 0.01$); m266-treated mice showing increased levels of soluble Aβ peptide. No significant differences in levels of Aβ were found between groups in the hippocampal extracts (data not shown). Interestingly, object recognition performance was also significantly correlated with levels of Aβ40 in CSF and in the cortical PBS-soluble pool ($r = 0.491$, $p < 0.05$ and $r = 0.605$, $p < 0.01$).

These results demonstrate that administration of an antibody having a very high affinity for soluble Aβ (but a very low affinity for insoluble Aβ) rapidly and efficiently reverses object recognition memory impairments in APPV717F transgenic mice. This rapid improvement in cognitive function is accompanied by significant and marked increases in plasma, CSF and cortical soluble levels of Aβ, but not by any measurable changes in the brain insoluble pool of Aβ.

Example 2

Rapid Effect of Administration of Anti-Aβ Antibodies on Cognition Correlated with Affinity for Soluble Aβ

The anti-Aβ murine antibodies 21F12 (recognizing Aβ42, but not Aβ40), 2G3 (recognizing Aβ40, but not Aβ42), 4G8 (binding Aβ between 13 and 28), 10D5 (recognizing 1-16), and 3D6 (binding 1-5) are administered to transgenic PDAPP mice as described above.

The performance of the mice administered these antibodies is then determined in the object recognition test as described above. Performance will correlate positively with the affinity of the antibody of soluble Aβ, that is, the higher the affinity of an antibody for

soluble Aβ, the generally higher will be the performance in tests of cognition within a short time after administering the antibody.

Antibody m266 causes more significant flux of Aβ into the plasma and faster, more complete recovery of object recognition than does an antibody such as 3D6, which has an affinity for soluble Aβ that is about 1,000-fold less than that of m266. Antibodies having higher binding affinity for soluble Aβ will cause more pronounced flux and significantly faster and better improvement in cognitive function.

Example 3

Spatial Learning in APPV717F Mice Following a Single anti-Aβ Antibody Treatment

The subjects were female APPV717F and wild-type mice approximately 11 months old. Each mouse was administered 355 μg of murine 266 antibody or vehicle (PBS) administered 24 hours prior to start of testing (i.p.) Mice were tested in a holeboard spatial learning assay for four consecutive days (Figure 3). Four holes were baited with access to a single food pellet and the remainder of the holes were baited beneath a screen without access. Mice were food-deprived each night before testing the next morning. Mice were tested for four, 180-second trials per day. Testing occurred for four consecutive days.

A single dose of Aβ antibody (266) significantly enhanced cognitive functioning of 11 month-old APPV717F mice compared to vehicle-treated transgenic mice (Figure 4).

A significant decrease in total errors was noted on Day 4 for vehicle-treated WT mice while the number of errors was similar across Days 3 and 4 for antibody-treated wild type mice (Figure 5).

Example 4

Effect of administration of various antibodies on plasma and cortical soluble Aβ concentrations after 24 hours

Transgenic (+/-) mice (4 months of age) were administered 355 μg of each antibody (intraperitoneal). Samples were obtained 24 hours later.

Table 1. Concentration of soluble Aβ42 in cortex (μg/g) 24 hours after administration of various anti-Aβ antibodies.

	Mean	S.E.M.
Control (5)	0.058	0.007
266 (4)	0.169	0.047
3D6 (5)	0.091	0.007
10D5 (5)	0.065	0.004

Table 2. Concentration (ng/mL) of Aβ in plasma samples 24 hours after administering various anti-Aβ antibodies.

	Aβ40		Aβ42	
	Mean	S.E.M.	Mean	S.E.M.
Control (4)	0.054	0.0045	0.064	0.004
266 (4)	5.0	0.13	9.2	0.055
10D5 (5)	0.19	0.022	0.20	0.002
Control (4)	0.50	0.02	0.13	0.005
3D6 (5)	3.0	0.35	1.18	0.15

Antibody 3D6 administration caused an increase in plasma Aβ40 as well as Aβ42 (6-fold, and 8-fold, respectively). Plasma Aβ40 and Aβ42 levels were increased by 10D5 administration as well (approx. 3-4 fold). Antibody 266 administration caused a very significant increase in both 40 and 42 (93-fold and 144-fold, respectively).

Soluble Aβ40 from cortical tissue was significantly increased by 266 only. Administration of 3D6 or 10D5 was without effect on soluble Aβ40 levels in brain.

In another study of the rapid effects of administration of antibodies, 355 μg of each of 266, 3D6, and 4G8 was administered ip to hemizygous PDAPP transgenic mice (3 months old). Samples were obtained 24 hours later.

Table 3. Concentration (ng/mL) of Aβ42 in plasma samples 24 hours after administering various anti-Aβ antibodies.

	Aβ42	
	Mean	S.E.M.
Control (7)	0.048	0.0018
266 (5)	3.8	0.30
4G8 (5)	0.23	0.035

Control (7)	0.088	0.0035
3D6 (5)	0.72	0.15

In another study of the rapid effects of administration of antibodies, 355 μg of each of anti-Aβ antibodies 2G3 (recognizing the C-terminus of Aβ40 but not Aβ42), 10D5 (recognizing the N-terminus of Aβ), and 21F12 (recognizing the C-terminus of Aβ42, but not Aβ40) was administered intraperitoneally. Samples were obtained 24 hours later. The only significant difference in plasma Aβ42 levels was in the 10D5 group.

Table 4. Concentration (ng/mL) of Aβ42 in plasma samples 24 hours after administering various anti-Aβ antibodies.

	Aβ42	
	Mean	S.E.M.
Control (5)	0.065	0.0055
2G3 (5)	2.34	0.091
10D5 (5)	0.087	0.0044
Control (5)	0.058	0.0029
21F12 (5)	1.80	0.036

Table 5. Concentration (μg/g) of soluble Aβ40 and Aβ42 in cortex 24 hours after administering various anti-Aβ antibodies.

	Aβ40		Aβ42	
	Mean	S.E.M.	Mean	S.E.M.
Control (5)	27	3	7.0	0.61
2G3 (5)	48	5	4.0	0.23
10D5 (5)	62	9	5.2	0.49
21F12 (5)	91	14	5.4	0.16

There were no significant differences in insoluble Aβ in any group. Administration of 21F12 and 10D5 resulted in a statistically significant increase in soluble Aβ40 levels in the brain. Soluble levels of Aβ42 were significantly decreased following treatment with all 3 antibodies.

In yet another study, administration of 360 μg of 266, 3D6, or 10D5 antibody per animal (5 animals per group, saline control) raised average plasma Aβ 1-40 approximately 334-, 92-, and 14-fold, respectively, and raised average plasma Aβ 1-42 approximately

168-, 32-, and 19-fold, respectively. Clearly the relative magnitudes of these responses are directly related to the relative affinities of the antibodies toward soluble Aβ.

Taken together, these studies of the effects of administration of anti-Aβ antibodies after 24 hours shows a strong relationship between the levels of plasma Aβ and the affinity of the antibody for soluble Aβ40 and Aβ42. Also, an antibody that causes a significantly higher flux of Aβ (i.e., 266 compared with 3D6) after 24 hours also causes a significantly higher recovery of cognition in the same period. Therefore, we believe that rapid improvement in cognition will be faster and/or greater in magnitude when an antibody having a higher affinity for soluble Aβ is administered.

Example 5

Cognition after administration of antibodies having a range of affinities for soluble Aβ

Antibodies having affinities for soluble Aβ between about 1 nM and about 1 pM are obtained or prepared as described herein. The antibodies are administered to transgenic mice as described above in Example 1. Antibodies having higher affinity for soluble Aβ will generally cause greater flux of Aβ within a short time after administration and also more greatly effect rapid improvement in cognition.

Example 6

Binding affinity for soluble Aβ

Antibody affinity for soluble Aβ is determined using a BIAcore biosensor 2000 and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-mouse Ig or anti-human Ig) is coupled via free amine groups to carboxyl groups on flow cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG is coupled to flow cell 1 as a background control. Test antibodies are captured to yield 300 resonance units (RU). Soluble Aβ 1-40 or 1-42 (Biosource International, Inc.) is then flowed over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the chip, bound anti-Aβ antibody is eluted from the chip using a wash with glycine-HCl (pH 2). A control injection containing no amyloid-beta serves as a control for baseline subtraction.

Sensorgrams demonstrating association and dissociation phases are analyzed to determine kd and ka. Using this method, the affinity of the following antibodies was determined for 1-42 and/or 1-40, and they are presented in Table 1. Two affinities were found for antibody 10D5.

Table 6. Affinity (nM) of various antibodies for soluble Aβ1-40 and/or soluble Aβ1-42.

	Soluble Aβ1-40	Soluble Aβ1-42
3D6	2.4	2.4
10D5	390	0.57/4,950
266	0.004	0.004
266 N56S*	-	0.0025
266 N56T†	-	0.0019
4G8	23	24
21F12	-	4.4
2G3	0.9	-

* deglycosylated 266 analog, wherein Asn at position 56 of the heavy chain variable region is replaced with Ser

† deglycosylated 266 analog, wherein Asn at position 56 of the heavy chain variable region is replaced with Thr

In Figure 6 is plotted log of flux vs. log affinity for soluble Aβ. Flux is defined as the fold increase in plasma Aβ (40 or 42) 24 hours after administration of antibody, as described in Example 4 above. Affinity is given in Table 2 above. Because 10D5 apparently had two affinities for soluble Aβ42 that varied in the extreme, only data on Aβ40 were used for 10D5. A distinct relationship between affinity for soluble Aβ and flux is evident.

In Figure 7 is plotted flux data against affinity for aggregated Aβ, using data taken from Bard, *et al., Nat. Med.* 6:916-919 (2000). Antibody 266 was reported not to bind to aggregated Aβ, and so no data are plotted for it. There is clearly no relationship between affinity for aggregated Aβ and flux.

As demonstrated herein, the affinity of anti-Aβ antibodies for soluble, not aggregated, Aβ is positively correlated with flux of Aβ from the brain into the plasma within 24 hours after administering the antibody. Furthermore, the rate of flux of Aβ is also related to acute improvement in cognitive performance as demonstrated in Example 1 and Figure 2. Antibodies having higher affinity for soluble Aβ will cause more pronounced flux and will more quickly and more significantly effect improvement in cognitive function in conditions and diseases involving Aβ. We conclusively demonstrate here that high affinity for soluble, not insoluble or aggregated, forms of Aβ is an important feature of anti-Aβ antibodies for rapidly treating cognitive impairment in Aβ related conditions and diseases. We also believe that high affinity for soluble, not insoluble or aggregated, forms of Aβ is an important feature of anti-Aβ antibodies for rapidly treating cognitive impairment in Aβ related conditions and diseases.

Example 7

Alternate procedure for determining binding affinity for antibodies having high affinity for soluble Aβ

The procedure is very similar to that used above in Example 6, with certain modifications as described below. Protein A or protein A/G is immobilized via amine coupling to flow cells 1 and 2 of a B1 or CM5 sensor chip (BIAcore). The test antibody is then captured in flow cell 2 at desired levels (usually a 10 – 60 second injection of antibody) and 5 minutes is allowed for the antibody to stabilize. An aliquot of frozen Aβ 1-40 solution is thawed and then diluted to make up the highest concentration (e.g., 200 nM), which is then serially diluted (1:2 dilutions) to the lowest concentration (e.g., 6.25 nM). Each concentration is injected over the surface for 5 minutes at a flow rate of 50 μL/min. To obtain an accurate measurement of off–rate for high affinity antibodies, the dissociation of Aβ 1-40 is followed for 5 hours. Aβ 1-40 and antibody are then eluted from both flow cells with a 40 second injection of glycine (pH 1.5). The signal is allowed to stabilize for 2 minutes before the next cycle. The data from flow cell 1 is subtracted from flow cell 2 to account for any bulk shifts due to buffer differences or non-specific binding to the sensor chip or protein A. The various concentrations are injected randomly and each concentration is run in duplicate. Two 0 nM runs are used to control for any

dissociation of antibody from the protein A or A/G capture surface. The data is analyzed using the Biaevalution software. A 1:1 model with mass transfer and a local Rmax is used as the best fit for the data.

Using this alternate method, the affinity of humanized 266 antibody for soluble Aβ 1-40 was found to be 0.4 M^{-9} (± 0.2 M^{-9}). Using this value of affinity, together with data for other antibodies (above), the log of flux vs. log affinity for soluble Aβ 1-40 was plotted (Figure 8). The very distinct relationship between affinity for soluble Aβ and flux continues to be evident.

Soluble Aβ 1-40 stocks and diluted samples are prepared as follows. Solid Aβ 1-40 (Biosource International, Camarillo California USA 93012) is dissolved to 1 mg/mL (about 230 μM) in water, and the solution is immediately aliquoted into 20 – 50 μL portions and then frozen (-70 °C). Alkaline conditions can be used to dissolve Aβ, as described by Fezoui, *et al., Amyloid:Int'l J. Exp. Clin. Invest.* 7:166-178 (2000). An alternate method for preparing aggregate-free soluble stock Aβ solutions is that of Zagorski, *et al.* [*Meth. Enzymol.* 309:189-204 (1999)]. This procedure involves, in sequence, dissolving the peptide in trifluoroacetic acid (TFA), evaporating the TFA, redissolving in hexafluoroisopropanol (HFIP), removing HFIP, and dissolving in water. Aliquoting and freezing may be performed either before or after removing HFIP. Stock Aβ solutions can be checked for aggregates by methods well known in the art, for example, light scattering [Tomski, *et al., Arch. Biochem. Biophys.* 294:630-638 (1992)], thioflavin T binding [LaVine, *Meth. Enzymol.* 309:274-285 (1999)], or Congo red binding [Klunk *et al., Anal. Biochem.* 266:66 (1999)]. Immediately before use, a stock aliquot is thawed and diluted to the highest concentration to be used (typically, about a thousand-fold dilution to about 200 nM). This 1000-fold diluted sample is expected to contain soluble Aβ that is predominantly monomeric. The great tendency of Aβ to self-associate means that even when carefully prepared as described, samples of Aβ may contain small proportions of Aβ dimer especially, and perhaps even some higher order association states. Stock aliquots are not refrozen after they have been thawed. Stock aliquots are not used after the day on which they are thawed, nor are diluted samples used after the day on which they are prepared.

Example 8

Effect of administration of various humanized antibodies on plasma Aβ concentrations in male cynomolgus monkeys after 24 hours

The animals (2 to 4 kg, young adult to adult, 2 animals per group) were given a bolus intravenous administration of antibody (1 mg/kg; humanized 266 - expressed in a host cell transformed to express SEQ ID NO:11 and SEQ ID NO:12; N56S - expressed in a host cell transformed to express SEQ ID NO:11 and SEQ ID NO:21; or humanized 3D6 - US 60/287,539, filed 2001 April 30). Animals were housed individually in stainless steel cages. Environmental controls were set to maintain 18 to 29 degrees Centigrade, a relative humidity of 30% to 70%, and a 12-hour light/12-hour dark cycle. They received certified primate diet one or two times daily. Water was provided ad libitum. The animals were selected for study based on body weights, clinical observations, clinical pathology data, and other data as appropriate. Plasma samples (pre-dose and 24 hours post-dose) were collected in EDTA and frozen until analysis.

Plasma concentrations of immunoreactive $A\beta_{1-40}$ or $A\beta_{-42}$ were determined using ELISA assays. Immunoreactive $A\beta_{1-40}$ was captured on the ELISA plate using mouse monoclonal antibody 2G3 or $A\beta_{1-42}$ was captured using mouse monoclonal antibody 21F12. The bound complex was detected using biotinylated-3D6 antibody, followed by addition of streptavidin-HRP. Color development was performed using TMB as a substrate. Optical density values were read at 450/630 nm, and raw data was analyzed with a 5-parameter logistic algorithm using STATLIA software (Brendan Scientific). Serum concentrations of immunoreactive $A\beta_{1-40}$ were estimated using calibrators ranging from 16 to 1000 pg/ml prepared in heat-treated, charcoal stripped human serum. Based on recovery of control samples and the back-fit of calibrators, the lower and upper limits of quantitation in this assay are estimated to be 50 and 1000 pg/mL, respectively. Results for Aβ1-40 are shown below in Table 7. Flux could not be calculated for Aβ1-42 because pre-dose concentrations were below the detection limit.

Table 7. Mean concentrations of immunoreactive antibodies and Aβ1-40 in cynomolgus monkeys (two animals per group).

humanized 266	N56S	humanized 3D6

$[A\beta 1\text{-}40]_{Pre\text{-}dose}$ (ng/mL)	0.6	1.4	0.6
$[A\beta 1\text{-}40]_{24\ hour}$ (ng/mL)	109	124	12.2
Difference (ng/mL)	108	123	11.6
Flux (-fold increase)	181	87.6	19.3

The increase in plasma Aβ1-40 24 hours after administration of humanized 266 and N56S was approximately the same, and much greater than the increase after administration of humanized 3D6. These data, obtained in normal monkeys (i.e., not having any known defect that affects Aβ metabolism or any known Aβ-related conditions or diseases), are consistent with the mouse studies described herein – that is, that the affinity of anti-Aβ antibodies for soluble, not aggregated, Aβ is positively correlated with flux of Aβ from the brain into the plasma within 24 hours after administering the antibody. N56S has a much faster turn-over than humanized 266 or 3D6 (5.5- and 8.6-times as much 266 and 3D6 in the plasma as N56S at 24 hours, though the doses were the same). Thus, the Aβ 1-40 levels at 24 hours for N56S are higher than those for humanized 266 when normalized to the concentration of antibody present, as would be expected on the basis of the present invention, because N56S has a higher affinity for soluble Aβ than humanized 266. Differences in flux caused by 266 and 3D6 cannot be attributed to differences in the pharmacokinetics for the two antibodies either in these monkeys or in the mice used in other experiments described herein.

We claim:

1. A method for effecting rapid improvement of cognition in a subject having a condition or disease related to Aβ, comprising administering to the subject an effective amount of an anti-Aβ antibody.

2. The method of Claim 1, wherein the subject is human.

3. The method of Claim 2, wherein the condition or disease is Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, or mild cognitive impairment.

4. The method of Claim 3, wherein the disease is Alzheimer's disease.

5. The method of Claim 3, wherein the disease or condition is Down's syndrome.

6. The method of Claim 3, wherein the disease or condition is cerebral amyloid angiopathy.

7. The method of Claim 3, wherein the disease or condition is mild cognitive impairment.

8. The method of any one of Claims 1 – 7, wherein the antibody has greater affinity for soluble Aβ than 10^{-9} M.

9. The method of any one of Claims 1 – 7, wherein the antibody has greater affinity for soluble Aβ than humanized antibody 266, which comprise SEQ ID NO:11 as the light chain and SEQ ID NO:12 as the heavy chain.

10. The method of any one of Claims 1 – 7, wherein the antibody has greater affinity for soluble Aβ than 10^{-10} M.

11. The method of any one of Claims 1 – 7, wherein the antibody has greater affinity for soluble Aβ than 10^{-11} M.

12. The method of any one of Claims 1 – 11, wherein the antibody is a humanized or human antibody.

13. The method of Claim 12, wherein the antibody is a humanized 266 antibody, or an analog thereof.

14. The method of any one of Claims 1 – 13, wherein the anti-Aβ antibody recognizes the same epitope that antibody 266 recognizes or competes with antibody 266 for binding to soluble Aβ.

15. The method of any one of Claims 1 – 14, wherein the affinity is measured with respect to either Aβ1 – 40 or Aβ1 – 42.

16. The method of any one of Claims 1 – 15, additionally comprising measuring cognition in the subject before administering the antibody.

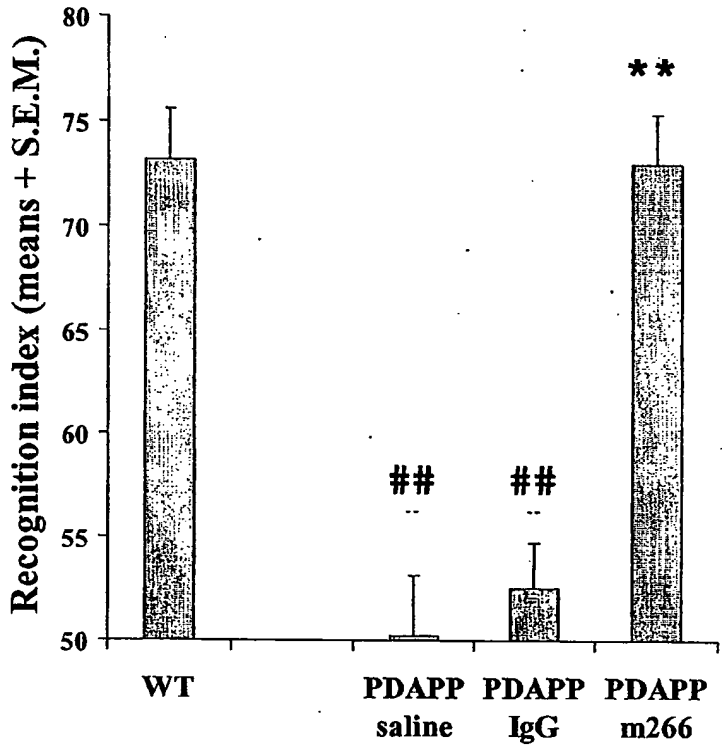
17. The method of Claim 16, additionally comprising measuring cognition in the subject after administering the antibody.

18. The method of Claim 17, wherein the measure of cognition after administering the antibody shows a significant improvement in cognition compared with the measure of cognition before administering the antibody.

19. The method of any one of Claims 1 – 18, additionally comprising measuring cognition in the subject after administrating the antibody.

20. The use of an anti-Aβ antibody to prepare a medicament for any one of the methods of Claims 1 – 19.

FIG. 1.


Recognition index (means + S.E.M.)
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75
70
65
60
55
50
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##
##
WT
PDAPP saline
PDAPP IgG
PDAPP m266

FIG. 2.

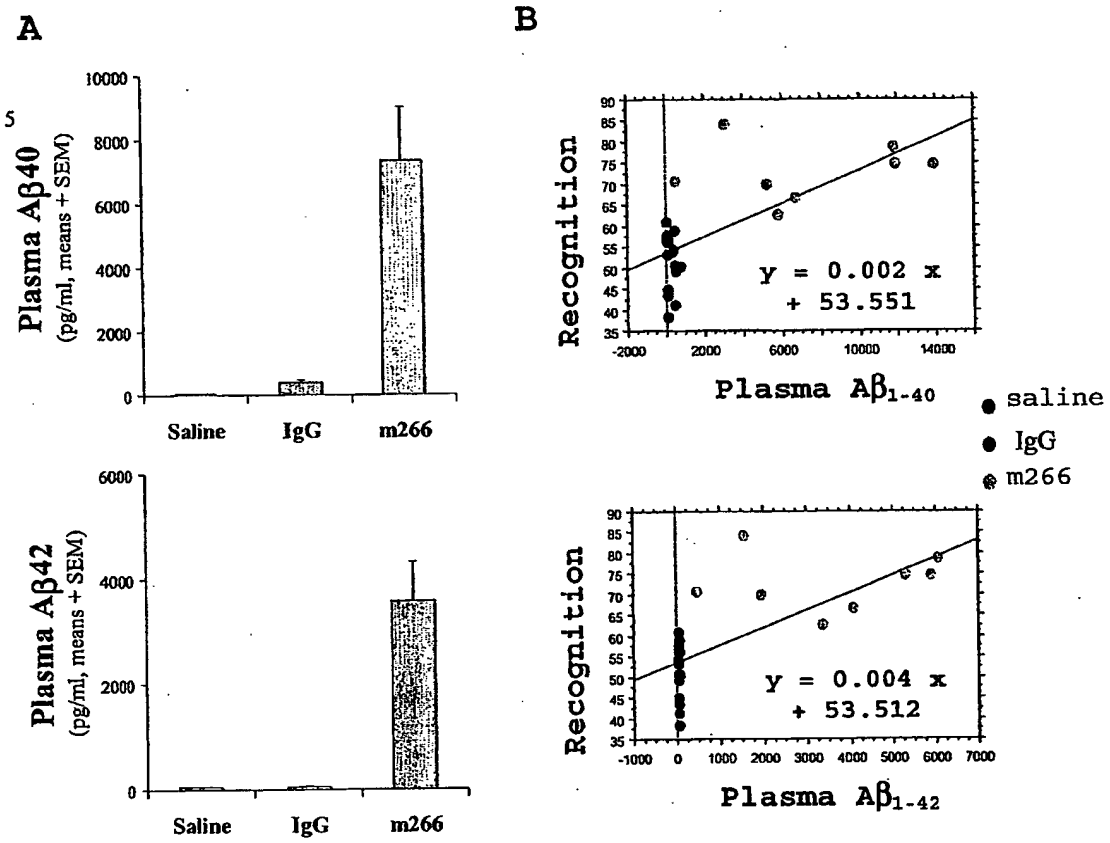

A
B
Plasma Aβ40
(pg/ml, means + SEM)
10000
8000
6000
4000
2000
0
Saline
IgG
m266
Plasma Aβ42
(pg/ml, means + SEM)
6000
4000
2000
0
Saline
IgG
m266
Recognition
y = 0.002 x
+ 53.551
Plasma Aβ1-40
saline
IgG
m266
Recognition
y = 0.004 x
+ 53.512
Plasma Aβ1-42

FIG. 3.

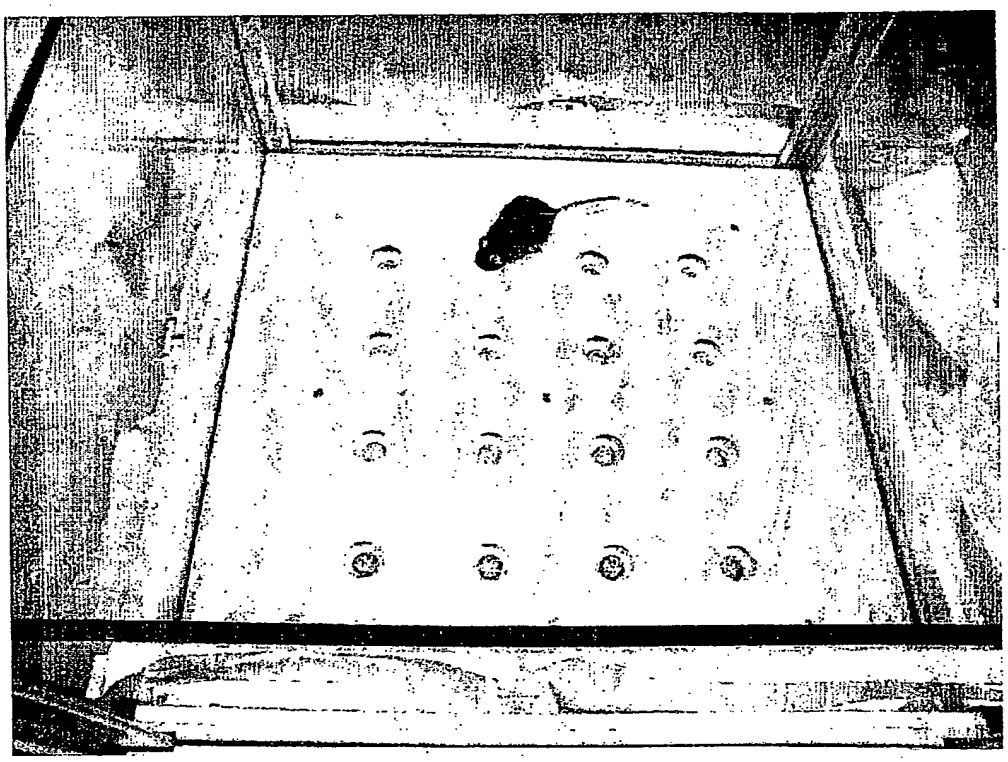


FIG. 4.

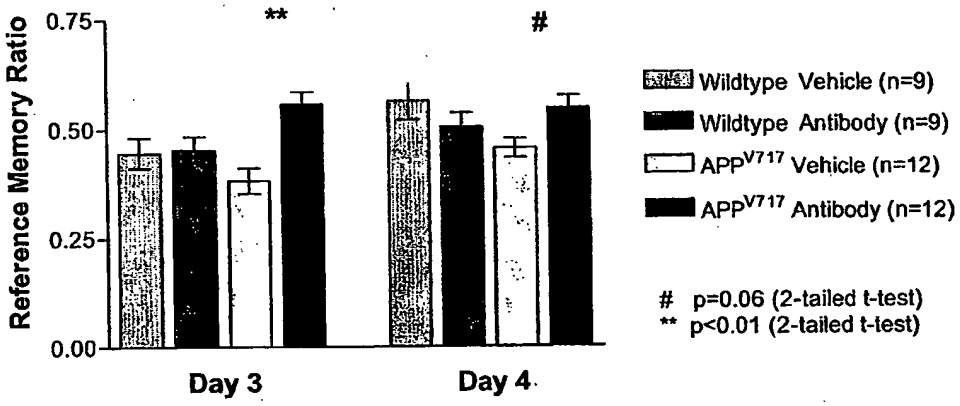
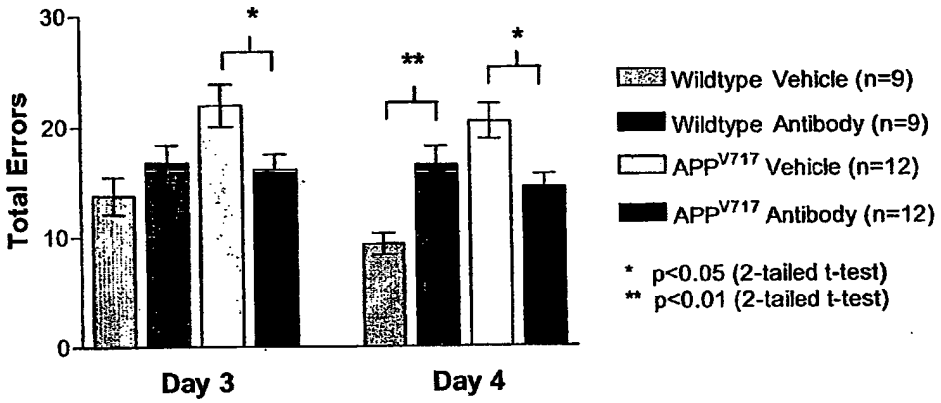

Reference Memory Ratio
0.75
0.50
0.25
0.00
**
#
Day 3
Day 4
Wildtype Vehicle (n=9)
Wildtype Antibody (n=9)
APP^{V717} Vehicle (n=12)
APP^{V717} Antibody (n=12)
p=0.06 (2-tailed t-test)
** p<0.01 (2-tailed t-test)

FIG. 5.


30
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10
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Total Errors
*
**
*
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Day 4
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Wildtype Antibody (n=9)
APP^{V717} Vehicle (n=12)
APP^{V717} Antibody (n=12)
* p<0.05 (2-tailed t-test)
** p<0.01 (2-tailed t-test)

FIG. 6.

5

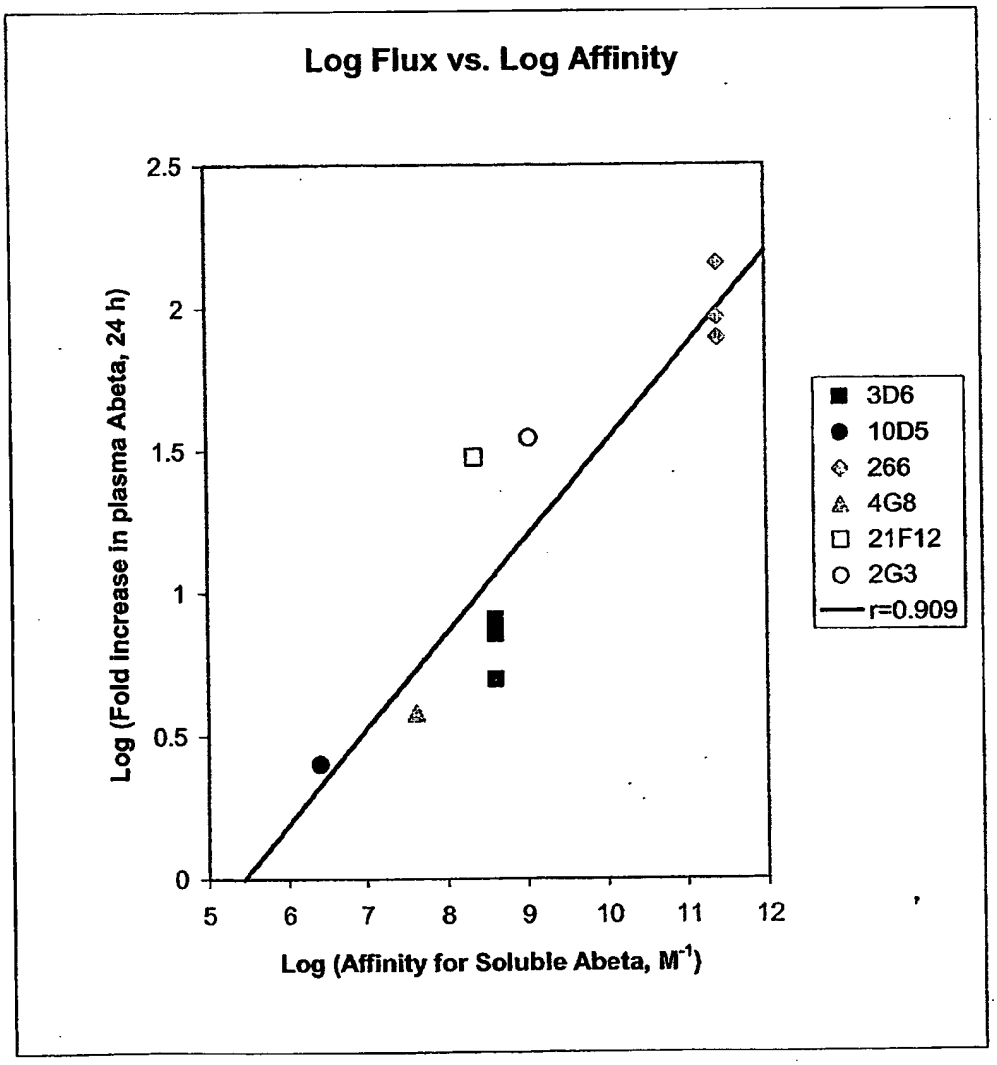

Log Flux vs. Log Affinity
Log (Fold Increase in plasma Abeta, 24 h)
Log (Affinity for Soluble Abeta, M-1)
2.5
2
1.5
1
0.5
0
5
6
7
8
9
10
11
12
3D6
10D5
266
4G8
21F12
2G3
r=0.909

FIG. 7.

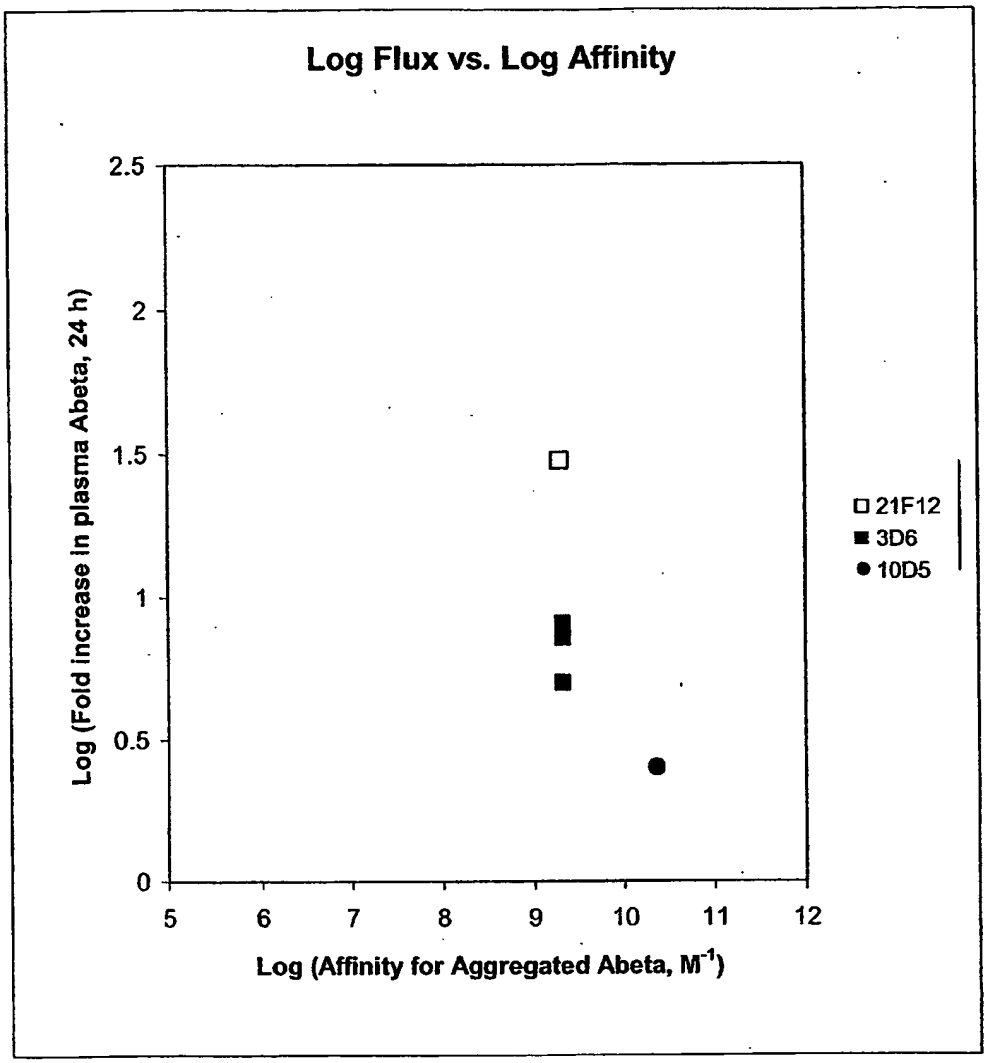
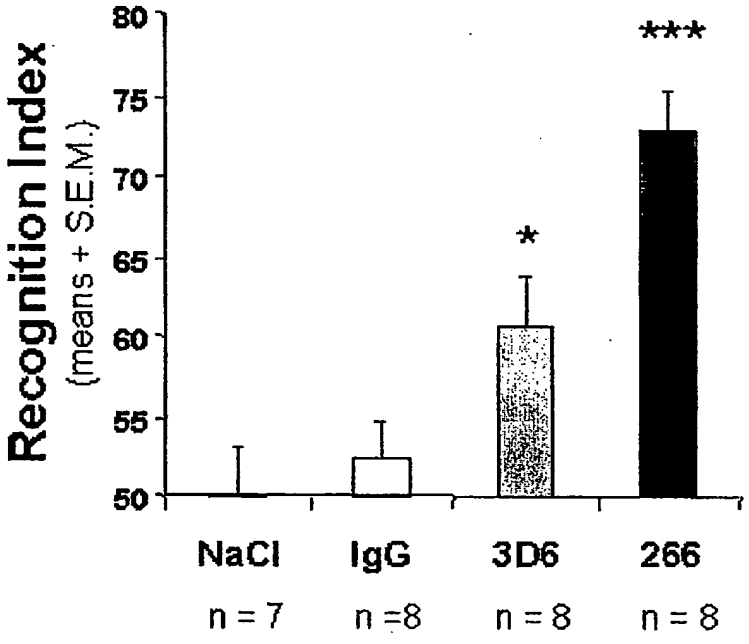

Log Flux vs. Log Affinity
Log (Fold increase in plasma Abeta, 24 h)
2.5
2
1.5
1
0.5
0
5
6
7
8
9
10
11
12
Log (Affinity for Aggregated Abeta, M-1)
21F12
3D6
10D5

FIG. 8.


Recognition Index
(means + S.E.M.)
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75
70
65
60
55
50
*
***
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3D6
266
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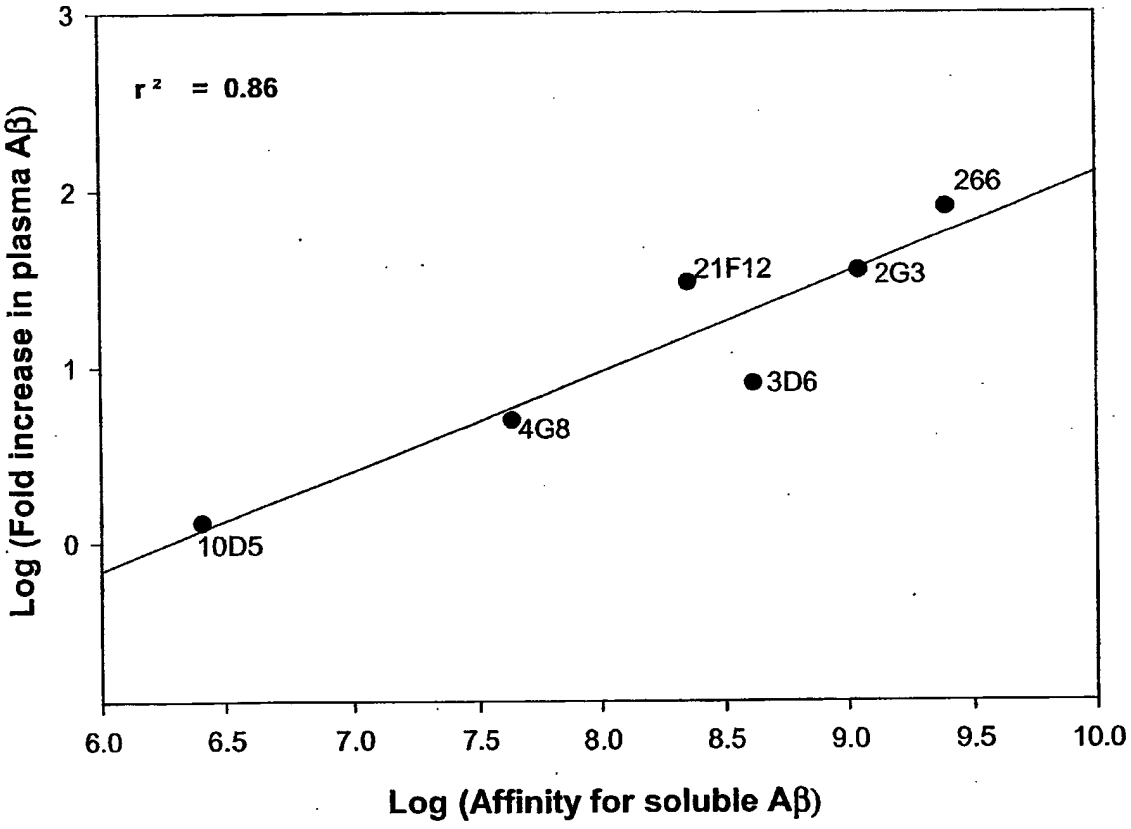
n =8
n = 8
n = 8

FIG. 9.


r² = 0.86
266
21F12
2G3
3D6
4G8
10D5
Log (Fold increase in plasma Aβ)
Log (Affinity for soluble Aβ)
3
2
1
0
6.0
6.5
7.0
7.5
8.0
8.5
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9.5
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<151> 2001-08-17

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<151> 2002-05-28

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Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

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20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
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<223> humanized antibody 266 preferred light chain variable

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Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile Tyr Ser
20 25 30

Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 10

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20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

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20 25 30

Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
        115                 120                 125

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
    130                 135                 140

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
145                 150                 155                 160

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
                165                 170                 175

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
            180                 185                 190

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
        195                 200                 205

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
    210                 215                 220

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
225                 230                 235                 240
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Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                245                 250                 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
            260                 265                 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        275                 280                 285

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    290                 295                 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305                 310                 315                 320

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                325                 330                 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
            340                 345                 350

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        355                 360                 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    370                 375                 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
385                 390                 395                 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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<222> (7)..(7)

<223> Xaa at position 7 is any amino acid, provided that if Xaa at position 8 is neither Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn

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1 5 10 15

Gly

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<223> heavy chain CDR2

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<223> Xaa at position 9 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr

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<223> Xaa at position 8 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr

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<222> (7)..(7)

<223> Xaa at position 7 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr

<400> 14

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 15

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp, and Tyr

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp, and Tyr

<400> 15

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 16

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Asp and Pro

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ser and Thr

<400> 16

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 17

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (1)..(17)

<223> heavy chain CDR2

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, G
ly, His, Asn, Gln, Ser, and Thr

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is selected from the group consisting of Ala,
Gly, His, Gln, Ser, and Thr

<400> 17

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 18

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, G
ly, His, Asn, Gln, Ser, and Thr

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, G
ly, His, Asn, and Gln

<400> 18

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 19

<211> 112

<212> PRT

<213> artificial sequence

<220>

<223> humanized antibody

<220>

<221> MISC_FEATURE

<222> (1)..(112)

<223> humanized antibody heavy chain variable

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at pos
ition 56 is Asn and Xaa at postion 57 is neither Asp nor Pro, the
n Xaa at position 58 is neither Ser or Thr

<220>

<221> MISC_FEATURE

<222> (57)..(57)

<223> Xaa at position 57 is any amino acid, provided that if Xaa at pos

ition 56 is Asn and Xaa at postion 58 is Ser or Thr, then Xaa at postion 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp or Pro and Xaa at position 59 is Ser or Thr, Then Xaa at postion 56 is not Asn

<220>

<221> MISC_FEATURE

<222> (107)..(107)

<223> Xaa is Leu or Thr

<220>

<221> MISC_FEATURE

<222> (89)..(89)

<223> Xaa is Glu or Asp

<220>

<221> MISC_FEATURE

<222> (76)..(76)

<223> Xaa is Lys or Arg

<220>

<221> MISC_FEATURE

<222> (75)..(75)

<223> Xaa is Ala, Ser, Val, or Thr

<220>

<221> MISC_FEATURE

<222> (63)..(63)

<223> Xaa is Thr or Ser

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<220>

<221> MISC_FEATURE

<222> (46)..(46)

<223> Xaa is Glu, Val, Asp, or Ser

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa is Ser or Leu

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> Xaa is Glu or Gln

<400> 19

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Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
            20                  25                  30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
        35                  40                  45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Xaa Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
            100                 105                 110
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<210> 20

<211> 112

<212> PRT

<213> artificial sequence

<220>

<223> humanized antibody

<220>

<221> MISC_FEATURE

<222> (1)..(112)

<223> heavy chain variable

<220>

<221> MISC_FEATURE

<222> (57)..(57)

<223> Xaa at position 57 is any amino acid, provided that if Xaa at pos ition 56 is Asn and Xaa at postion 58 is Ser or Thr, then Xaa at postion 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at pos ition 56 is Asn and Xaa at postion 57 is neither Asp nor Pro, the n Xaa at postion 58 is neither Ser or Thr

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid, provided that if Xaa at pos ition 57 is neither Asp nor Pro and Xaa at postion 59 is Ser or T hr, then Xaa at position 56 is not Asn

<400> 20

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 21

<211> 442

<212> PRT

<213> artificial sequence

<220>

<223> humanized antibody

<220>

<221> MISC_FEATURE

<222> (1)..(442)

<223> heavy chain antibody

<220>

<221> MISC_FEATURE

<222> (57)..(57)

<223> Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at postion 58 is Ser or Thr, then Xaa at postion 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

<400> 21

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
            20                  25                  30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
        115                 120                 125

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
    130                 135                 140

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
145                 150                 155                 160

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
```

165 170 175

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
180 185 190

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
195 200 205

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
210 215 220

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
225 230 235 240

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
245 250 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
260 265 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
275 280 285

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
290 295 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305 310 315 320

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
325 330 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
340 345 350

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
355 360 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
370 375 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
385 390 395 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
405 410 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr

420 425 430

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440